Multiscale Modeling of Circadian Rhythms

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Overview

We report the computational prediction of regulation, metabolite levels and rate constants using a maximum entropy method [1], and the experimental detection of circadian regulation of proteins and transcripts [2]. The computational method is applied in four steps: (1) a new constrained optimization approach is used to obtain the maximum entropy distribution, (2) the predicted metabolite concentrations are compared to those generally expected from experiment using a loss function from which post-translational regulation of enzymes is inferred, (3) the system is re-optimized with the inferred regulation from which rate constants are determined from the metabolite concentrations and reaction fluxes, and finally (4) a full ODE-based, mass action simulation with rate parameters and allosteric regulation is obtained. The method is applied to central metabolism and the flow of material through the three competing pathways of upper glycolysis, the non-oxidative pentose phosphate pathway, and the oxidative pentose phosphate pathway are evaluated as a function of the NADP/NADPH ratio.

To complement the simulations, experimental transcriptional and translational experiments performed over the circadian cycle of *Neurospora*. Transcriptional/translational feedback loops in fungi and animals drive circadian rhythms in transcript levels that provide output from the clock, but post-transcriptional mechanisms also contribute. We applied novel analytical tools to a long-duration, deeply-sampled, circadian proteomics time course comprising half of the proteome. The experimental data reinforces our simulations and demonstrate that the rhythmic proteins within the Pentose-Phosphate pathway peak in the circadian morning, while conversely, in glycolysis and the TCA cycle, the rhythmic proteins peak in the circadian evening. That is, the rhythmic proteins of glycolysis are in anti-phase to the rhythmic proteins of the Pentose-Phosphate pathway.

Prediction of Dynamics & Regulation

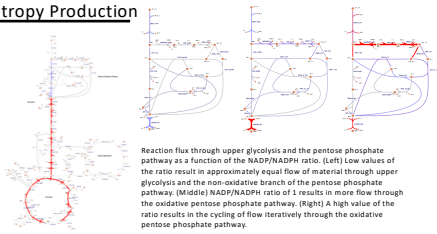
The new approach to the law of mass action does not require rate parameters but instead uses chemical potentials (1). Due to the statistical formulation of the theory, the approach can directly integrate metabolomics and proteomics data.

Modeling Approach

◆ Optimization and Maximum Entropy Production

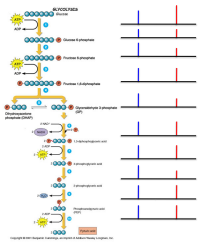
$$\frac{dn_i}{dt} = \sum_{\alpha}^{Z} c_\alpha \frac{1}{\gamma_{i,\alpha}} \left(e^{A_\alpha(n_i)/RT} - e^{-A_\alpha(n_i)/RT} \right)$$

The Marcelin Equation sets each of the decay rates of the forward and reverse forces to the same rate. Assuming the same decay rate for *all* reactions removes any kinetic bottlenecks in phase space of the system such that the dynamics are governed only by the thermodynamics



Reaction flux through upper glycolysis and the pentose phosphate pathway as a function of the NADP/NADPH ratio. (Left) Low values of the ratio result in approximately equal flow of material through upper glycolysis and the non-oxidative branch of the pentose phosphate pathway. (Middle) NADP/NADPH ratio of 1 results in more flow through the oxidative pentose phosphate pathway. (Right) A high value of the ratio results in the cycling of flow iteratively through the oxidative pentose phosphate pathway.

◆ Reinforcement Learning of Regulation



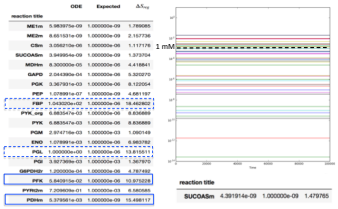
Experiment
Simulation

Adjust enzyme activity until the simulation and experimental measurement agree

$$\Delta S_{reg} = \sum_{j}^{k} \log\left(\frac{\theta_j}{\rho_j}\right)$$

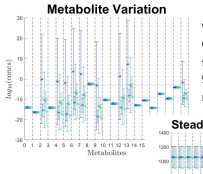
Loss Function Before Regulation

Metabolite Concentrations



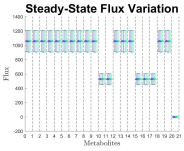
◆ Uncertainty Quantification

Metabolite Variation



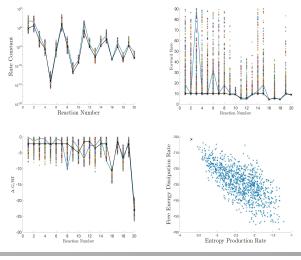
Variation in flux and Concentrations due to variation in $K\alpha$ in 95% confidence range.

Steady-State Flux Variation



$$K_\alpha Q_\alpha^{-1} = e^{A_\alpha(n_i)/RT}$$

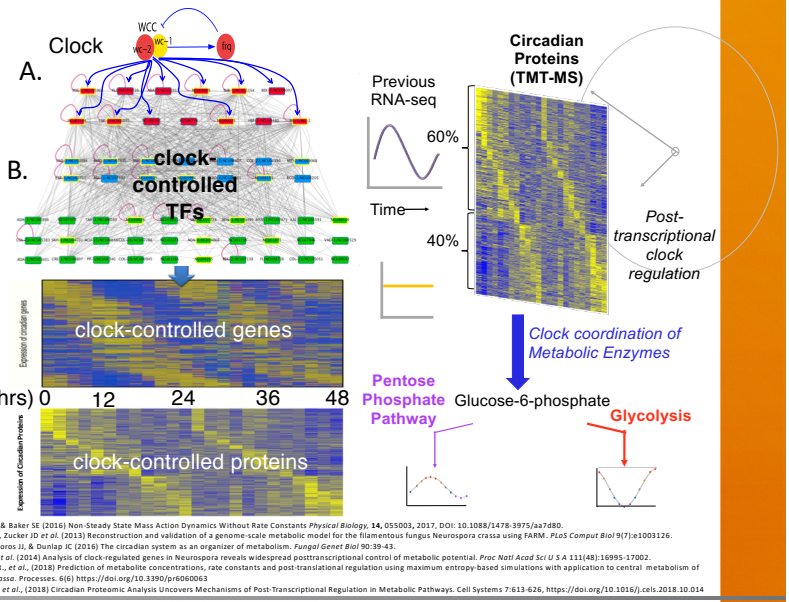
Relaxing the Assumption of Maximum Entropy: 1000 Models in ~30 min.



Observation of Dynamics & Regulation

Lower Left: The circadian cycle is approximated in (**A**) by the negative feedback loop in which the heterodimer WC-1/WC-2 drives expression of *frq* which feeds back with other proteins (not shown) to depress WC-1/WC-2 activity. In (**B**), WC-1/WC-2, in turn, activate clock-controlled TFs (curved blue arrows) and these in turn regulate additional TFs, in all comprising a hierarchical network downstream from the clock. This transcriptional network, now largely described from ChIP-seq data for over 50 TFs, acts as a dynamic filter for time information generated by the circadian oscillator in (**A**). In the aggregate the TFs within this transcriptional network act on downstream genes in a combinatorial manner to regulate their expression. Shown in (**C**) is the heat map showing rhythmic expression of the *Neurospora* genome as determined by RNA-seq of samples collected every 2 hrs over 48 hrs in constant darkness., and in (**D**) the corresponding map for proteins whose expression is controlled by the clock.

Lower Right A quarter of expressed proteins are clock-regulated, but >40% of these do not arise from clock-regulated transcripts. Contrary to predictions, rhythmic protein degradation plays little role in posttranscriptional regulation but instead rhythms arise from oscillations in translation. Our data highlighted the impact of the clock on metabolic regulation, with central carbon metabolism reflecting both transcriptional and posttranscriptional control and opposing metabolic pathways showing peak activities at different times of day. The experimental data demonstrate that the rhythmic proteins within the Pentose-Phosphate pathway peak in the circadian morning, while conversely, in glycolysis and the TCA cycle, the rhythmic proteins peak in the circadian evening. That is, the rhythmic proteins of glycolysis are in anti-phase to the rhythmic proteins of the Pentose-Phosphate pathway.



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